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(54) Title: PRECIPITATION OF COLLAGEN IN TACTOID FORM

(57) Abstract

Collagen in tactoid form obtained by forming an aqueous solution containing dissolved collagen and a water soluble or miscible polymer adapted to precipitate collagen out of solution in the form of tactoids.

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PRECIPITATION OF COLLAGEN IN  
TACTOID FORM

1           This invention relates to collagen products.  
2           In a particular aspect this invention relates  
3         to collagen products made from soluble collagen. A new  
4         method by which soluble collagen can be formed into  
5         quasi-crystalline structures by precipitation using  
6         soluble polymers is described. The use of an aggregate  
7         of this quasi-crystalline collagen to form a variety of  
8         collagen materials which have improved properties  
9         compared with existing collagenous materials is  
10       described. Such improved collagen materials have  
11       application in various fields including the manufacture,  
12       for example, of products for medical use.

13           Collagen is an extremely common protein in the  
14       animal kingdom and therefore many uses for products  
15       based upon collagen have developed. Many products use  
16       collagen in either its native form (i.e. the triple  
17       helical structure pre-existing in an animal or human  
18       body), or regenerated into this form, or after denaturation  
19       of the collagen, in the form of gelatine. Native collagen  
20       is used for various products such as in the production  
21       of leather from animal skins, or such as the production  
22       of sausage casings in which the collagen is finely  
23       divided and reformed into the desired structure.

24           There are also many uses of collagen and for items  
25       made from collagen in medical fields such as in  
26       artificial arteries, veins, tendons, corneas, heart  
27       valves, skin, or patches or the like which are used as  
28       replacement parts for disease or injury affected parts in  
29       humans, or in cosmetic applications such as mammary  
30       prostheses or injectable collagen, or in collagen  
31       sponges, sutures or haemostat materials which may be  
32       used during surgery or in the treatment of disease  
33       (Chvapil, 1979). Many of these medical products made from  
34       collagen are at present unsatisfactory because of an  
35       inability to reproduce the native structure, composition  
36       or strength which exists in the normal  
37       collagenous tissue or because of the immune response

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1 elicited by the presence of immunogenic collagen or  
2 components or other material foreign to the body.

3 In its native form in the body, collagen exists in  
4 many types and in the most common of these types, collagen  
5 exists as fibrils in which individual collagen  
6 molecules are arranged in a staggered overlap  
7 structure (Bornstein and Traub, 1979). These fibrils  
8 are stabilised and made insoluble by  
9 intermolecular crosslinks between the non-helical  
10 portions (telopeptides) of adjacent collagen molecules  
11 (Bornstein and Traub, 1979). If the collagen from normal,  
12 mature tissue is to be made soluble the crosslinks must  
13 be broken, for example by digestion with an enzyme such as  
14 pepsin.

15 Soluble collagen can be reconstituted in a variety  
16 of ordered aggregate forms. Some are fibrous in form,  
17 and fibrils in which the collagen is arranged in its  
18 native staggered way can be reformed. The rate of the  
19 fibril reforming process is enhanced if collagen with  
20 intact telopeptides is used. However, results from the  
21 use of injectable soluble collagen have shown that the  
22 telopeptides lead to an antigenic response in humans;  
23 collagen lacking telopeptides is relatively non antigenic  
24 (Linsenmayer, 1982) but can still be made to form fibrils.  
25 Materials formed by fibril regeneration are often too  
26 hydrated and additional methods such as freezedrying or  
27 cell-induced contraction must be used to give a functional  
28 product.

29 Other non-native fibrous aggregates, termed  
30 FLS collagen, can be formed in which the collagen molecules  
31 are arranged in various staggered arrangements with  
32 the orientation of the molecules in both directions.

33 Quasi-crystalline aggregates can also be formed.  
34 These include very small crystallites of collagen,  
35 termed SLS collagen, in which the collagen molecules all  
36 have the same orientation, but there is no stagger  
37 between molecules. These have been of partial use in  
38 deducing the native structure of collagen but SLS collagen

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1 has been of little use in the manufacture of larger  
2 structures like biomedical products. Also, quasi-  
3 crystalline tactoids of collagen can be prepared, using  
4 conditions similar to those used for reconstituting  
5 fibrils by heat gelation (Leibovich and Weiss, 1970; Lee  
6 and Piez, 1983) but the technique of production is more  
7 difficult than the technique described here as it  
8 does not involve simple precipitation. In these  
9 structures the collagen is arranged in a staggered form  
10 similar to native fibrils. In the present work the  
11 tactoids are produced by a new procedure,  
12 precipitation by soluble, neutral polymers. When collagen  
13 is precipitated by other procedures, for example salts,  
14 alcohols or heat, amorphous precipitates are formed.

15           DESCRIPTION OF THE INVENTION

16           During a search for more efficient methods of  
17 isolating soluble collagen it was found that the addition of  
18 water soluble polymers to a solution of collagen resulted  
19 in an efficient precipitation of the collagen from  
20 solution and the precipitated collagen was found to be much  
21 easier to separate from the liquid phase than with  
22 precipitates of collagen formed by the use of salts,  
23 alcohol or heat. The polymers had other advantages when  
24 compared with these previously used precipitants  
25 including that they were non-denaturing and did not  
26 require removal prior to chromatography or  
27 electrophoresis.

28           It was an unexpected finding that the collagen  
29 had precipitated in the form of small, needle-  
30 like, quasi-crystalline tactoids which were visible under  
31 the light microscope.

32           It was a further unexpected discovery that the  
33 tactoids could be induced to form into larger assemblages  
34 either by allowing the suspension to mature for a period  
35 of time or by mechanical action, and that the tactoids or  
36 their assemblages could be formed into shapes.

37           Accordingly, the present invention provides a method  
38 of producing a collagen product comprising forming an

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1 aqueous solution containing dissolved collagen and a water  
2 soluble or miscible polymer adapted to precipitate the  
3 collagen out of solution in the form of tactoids.

4 The pH of the said solution is preferably 3.5-10  
5 more preferably 5-8 with 7-8 being still more preferred and  
6 about 7.5 being most preferred.

7 The collagen precipitate may be left in the form of  
8 a paste or slurry and used in this form or after  
9 concentration by any one of the methods gravitational  
10 precipitation, filtration, centrifugation or the like. The  
11 precipitate may be crosslinked, tanned or stabilised by  
12 one or more of chemical, physical or biochemical methods  
13 either before or after it has been concentrated.  
14 Crosslinking, tanning or stabilisation applied to the  
15 precipitate before concentration makes the tactoids  
16 resistant to deforming actions such as heating,  
17 pressure or biochemical degradation. Crosslinking,  
18 tanning or stabilisation applied to the precipitate  
19 after concentration causes the structure formed  
20 during the concentration process to become more stable.

21 The so precipitated collagen may also be formed,  
22 for example, into a synthetic body part. Such forming  
23 into a synthetic body part may be effected by  
24 gravitational precipitation, filtration, centrifugation,  
25 moulding, pressing, shaping or any other way or combination  
26 of ways.

27 Shapes which may be prepared include sheets,  
28 tubes, strings and rods.

29 It has been found particularly desirable to form the  
30 so precipitated collagen into sheets for use as  
31 synthetic dressings for wounds and into tubes for use as  
32 synthetic tubular body parts. The sheets can be  
33 formed by centrifugation in a large basket centrifuge or  
34 the like or by gravitational precipitation or filtration.  
35 Other methods of producing the sheets are also possible.  
36 A more compacted sheet is produced by centrifugation  
37 in comparison with gravitational precipitation or  
38 filtration. Tubes can also be prepared by centrifugation

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1 or by casting, moulding or shaping.

2 The collagen may be precipitated onto a  
3 suitable substrate to form a composite material. Such a  
4 substrate, onto which the collagen is precipitated, may have  
5 the form of a particular body part or biomedical product.

6 The substrate may take the form of a matrix.

7 The substrate may take the form of a plastic or  
8 other synthetic surface in the form of a sheet, tube or  
9 mesh, onto which the collagen is directly deposited  
10 forming a collagenous coating.

11 The substrate may also take the form of a composite,  
12 for example, various synthetic layers bonded to an  
13 artificially or naturally-produced matrix.

14 These collagen coated substrates may also be  
15 chemically modified. For example, glutaraldehyde or  
16 similar chemicals may be used to stabilise the matrix.

17 The collagen of the present invention may be used as a  
18 paste or slurry. Such a paste or slurry would have a number  
19 of applications including as an implant material such as in  
20 the form of an injectable medium for use in cosmetic  
21 surgery. Such a slurry may be stabilized chemically such as  
22 by glutaraldehyde or irradiation. Such as with gamma  
23 radiation. The concentration of this tactoidal collagen in  
24 the paste or slurry is preferably not less than 10 mgm/ml,  
25 more preferably not less than 30 mgm/ml and most preferably  
26 not less than 40 mgm/ml.

27 The collagen useful for forming the collagen products  
28 of this invention includes collagen derived from hides,  
29 skins or other collagen containing organs or tissues of  
30 humans or other vertebrates or invertebrates and includes  
31 collagens of one type or mixtures of types. Soluble  
32 collagen can be prepared by enzymic treatment of collagen  
33 from those sources. Suitable enzymes include pepsin.

34 The collagen may also be derived from the culture  
35 medium of cells, tissues or organs grown in cell- or tissue-  
36 culture. The culture medium used to produce the collagen  
37 may be a culture medium from cell or tissue culture  
38 derived from a person for whom a synthetic body part is

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1 to be produced; it is believed that doing this will  
2 substantially reduce the likelihood of rejection.  
3 Further, it is also possible that a substrate may be  
4 introduced into the culture medium such that collagen and  
5 other components will be directly produced thereon.  
6 Such a substrate may have the form of a particular body  
7 part or biomedical product desired. The substrate may  
8 take the form of a matrix. The substrate may take the form  
9 of a plastic or other synthetic surface in the form of a  
10 sheet, tube or mesh, onto which the collagen and other  
11 components are directly deposited forming a collagenous  
12 coating. The substrate may be formed from aggregates of  
13 tactoidal collagen of this invention.

14 The water soluble or miscible polymer is preferably  
15 a neutral polymer. Such polymers may be at least one of  
16 the synthetic polymers polyvinyl alcohol, polyethylene  
17 oxide, polyvinylpyrrolidinone, polyacrylamide, polyethylene  
18 glycol, polypropylene glycol, polyvinyl methyl ether,  
19 maleic anhydride copolymers and the like; or at least one  
20 of the modified, natural, neutral polymers hydroxyethyl  
21 starches, methyl cellulose, hydroxymethyl cellulose,  
22 hydroxyethyl cellulose, hydroxypropyl cellulose or the like;  
23 or at least one of the natural neutral polymers  
24 agarose, dextrins, dextrans, starches, pectins,  
25 alginates and the like. Mixtures of such polymers  
26 may be used and the molecular weight of the polymer  
27 or polymers can vary over a wide range provided the  
28 polymer remains soluble or miscible with water.

29 This list of polymers is not exhaustive as the  
30 important factor is the use of a water soluble polymer or  
31 polymers to precipitate the collagen. Neutral water  
32 soluble or miscible polymers are preferable but charged,  
33 water soluble polymers may also be used particularly  
34 if they are only mildly charged.

35 The precipitate of collagen is generally found to  
36 be improved if it is allowed to stand in said solution.  
37 Such standing is preferable for a period of one hour to six  
38 months with one day to one month being more preferred.

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1        Such standing is effected at temperatures between  
2    the denaturation temperature of the collagen and the  
3    freezing point of the solution; preferably at between zero  
4    and 20°C; more preferably between zero and 10°C.

5        If desired, added materials such as  
6    plasticisers, colourants, biologically active  
7    materials such as proteoglycans or  
8    glycosaminoglycans, proteins, other extracellular  
9    products, hormones, growth factors, antibiotics and agents  
10   which affect wound healing or have other beneficial  
11   effects, ionic strength modifiers such as salts, or  
12   solids such as insoluble collagen or the like may be  
13   included with the so precipitated collagen and  
14   incorporated into material made from the collagen. These  
15   added materials may also be incorporated into the  
16   solution of soluble collagen before addition of the  
17   polymer or otherwise incorporated into material made  
18   from the collagen. Charged, water soluble or water  
19   miscible polymers may be used as part of a mixture with  
20   the neutral polymer or polymers and added to the soluble  
21   collagen with the neutral polymer solution. These  
22   charged polymers may be used to modify the properties of  
23   the soluble collagen solution or the material made from  
24   the precipitated collagen.

25       The collagen product of this invention may be  
26   chemically or biochemically stabilised. Biochemical  
27   stabilisation may be effected by enzymes such as  
28   lysyl oxidase. Chemical stabilisation may be effected  
29   by tanning agents, syntans, other cross-linking agents  
30   or chemical modifiers of collagen. Of particular  
31   interest are stabilisers which limit proteolysis  
32   or the immunogenicity of the collagen.  
33   Glutaraldehyde is a stabiliser of particular interest.  
34   The product may also be stabilised by dehydration by mild  
35   heat, water miscible solvents, critical point drying or the  
36   like. Such stabilisation may be performed before or after a  
37   shaping operation. The collagen product of this  
38   invention may be sterilised chemically or by irradiation.

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1 Chemical sterilisation may be conducted by means of  
2 suitable solutions of sterilising materials such as  
3 glutaraldehyde from between 0.5% to 5% concentration.  
4 The product may be stored in solutions of sterilant  
5 until required for use. Sterilisation by means of  
6 irradiation can be conducted by exposing the collagen  
7 product of this invention to gamma rays from a suitable  
8 source. From 0.5 to 5 Mrads of irradiation may be used,  
9 preferably 2.5 Mrads of gamma ray irradiation is suitable  
10 for satisfactory sterilisation of the product.

11 The tactoids formed by precipitation of the  
12 soluble collagen in this invention are useful in  
13 production of synthetic body parts, and other materials  
14 for medical or veterinary applications. The collagen  
15 tactoids or tactoid assemblages could be stabilised by  
16 chemical or biochemical techniques or could be formed  
17 into various useful shapes and then stabilised. The  
18 tactoidal collagen has potential application in many  
19 areas such as the manufacture of collagen sponges or  
20 haemostatic agents, of dressings, of membranes, of skin,  
21 of tubes and the like and in the treatment of  
22 disease such as periodontal disease. The tactoidal  
23 collagen can also be used in conjunction with other  
24 structural type materials to form composite materials  
25 with different properties. For example, a tube of  
26 tactoidal collagen can be covered with a woven or knitted  
27 mesh of fibre such as Dacron to give the tube additional  
28 strength. Alternatively, the tactoidal collagen can be  
29 formed into a tube surrounding the mesh to give a more  
30 intimate contact with the mesh and better properties. To  
31 better utilise the properties of the tactoidal  
32 collagen in the formation of artificial body parts it is  
33 possible to arrange the tactoids in a preferred  
34 orientation by the application of an electric field or  
35 by means of mechanical action. Materials made from the  
36 oriented tactoids may have beneficial effects in the  
37 healing of wounds. Many other methods of utilising  
38 the tactoidal collagen in a variety of shapes and forms

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1 and in conjunction with diverse other materials can be  
2 envisaged.

3 The product of this invention also has application  
4 in areas outside medical and veterinary products  
5 including plastics, fabric, leather or as composites or the  
6 like.

7 The present invention also includes such  
8 collagen products and articles produced therefrom.

9 The collagen products of this invention have  
10 advantages over presently available products. These  
11 include, low immunogenicity, ease of preparation, high  
12 collagen content, and strength.

13 The following examples illustrate the invention.

14 EXAMPLE 1

15 Type I collagen was solubilised and extracted from  
16 foetal calfskin by pepsin digestion and purified by  
17 fractional salt precipitation according to the method  
18 of Trelstad et al.(1967). This purified collagen was  
19 dissolved in 200 mM Tris-HCl buffer pH 7.5 at 4°C and at  
20 a concentration of 10 mg/ml. Polyethylene glycol (PEG)  
21 4000 was then added to produce a final concentration of  
22 2.5% (w/v). A precipitate of tactoidal collagen formed  
23 which settled to the bottom of the container after  
24 standing at 4°C for a few hours or could be concentrated  
25 by filtration or centrifugation.

26 EXAMPLE 2

27 As for Example 1 except that the concentration  
28 of the collagen was 1 mg/ml.

29 EXAMPLE 3

30 As for Example 2 except that PEG 400 to a final  
31 concentration of 3.5% (w/v) was used to precipitate the  
32 collagen.

33 EXAMPLE 4

34 Type III collagen, solubilised and extracted as in  
35 Example 1, was dissolved at a concentration of 1 mg/ml in  
36 200mM Tris- HCl buffer pH7.6 at 4°C. PEG 400 was added to  
37 the solution to a final concentration of 4.0% (w/v) and  
38 the precipitate of tactoidal collagen formed.

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1           EXAMPLE 5

2           As for Example 4 except that a final concentration of  
3   2.5% (w/v) PEG 4000 was used.

4           EXAMPLE 6

5           Type II collagen was isolated by the method of  
6   Trelstad et al. (1976) from bovine articular  
7   cartilage by pepsin solubilisation and fractional  
8   salt precipitation. The purified type II collagen was  
9   dissolved in 200 mM Tris-HCl buffer at pH 7.6 at 4°C and  
10   at a concentration of 1 mg/ml. PEG 400 was then added to  
11   produce a final concentration of 3.0% (w/v). The  
12   precipitate of tactoidal collagen formed as in Examples  
13   above.

14          EXAMPLE 7

15          As for Example 6 except that PEG 4000 was added to a  
16   final concentration of 2.0% (w/v).

17          EXAMPLE 8

18          As for Example 1 except that PEG 1000 to a  
19   final concentration of 5% (w/v) was used to  
20   precipitate the collagen.

21          EXAMPLE 9

22          As for Example 1 except that PEG 10000 to a  
23   final concentration of 5% (w/v) was used to  
24   precipitate the collagen.

25          EXAMPLE 10

26          The suspension of tactoidal collagen from Example  
27   1 was stored at 4°C for 4 weeks and collected on  
28   Whatman No. 1 filter paper in a 125 mm diameter basket  
29   centrifuge rotating at 4000 rpm. The resulting collagen  
30   sheet was removed from the centrifuge and separated from  
31   the filter paper. The collagen sheet was found to have  
32   properties similar to those of a thick, wet paper tissue  
33   and to be suitable for assisting in the healing of open  
34   skin wounds.

35          EXAMPLE 11

36          The collagen sheet, prepared as in Example 10, was  
37   tanned using a solution of 0.01% glutaraldehyde for 18  
38   hours. After drying the sheet was found to have a

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1 tensile strength of 6.2N/sq cm and an elongation of 12%  
2 at a moisture content of 16%.

3 EXAMPLE 12

4 The collagen sheet, prepared as in Example 10 was  
5 sealed in a polyethylene bag and subjected to 2.5Mrads  
6 of gamma ray irradiation. The sheet was found to have  
7 been sterilised and to have improved tensile properties  
8 over those of the sheet in Example 10.

9 EXAMPLE 13

10 As for Example 2 except that the buffer was at pH5.

11 EXAMPLE 14

12 As for Example 1 except that the collagen extracted  
13 from foetal calfskin was not purified by fraction  
14 salt precipitation but was used as a crude extract and that  
15 5% PEG 4000 was used.

16 EXAMPLE 15

17 As for Example 14 except that 5% polyvinyl alcohol was  
18 used.

19 EXAMPLE 16

20 As for Example 14 except that 5% dextran of 10,000  
21 average molecular weight was used.

22 EXAMPLE 17

23 As for Example 14 except that 5% dextran of 40,000  
24 average molecular weight was used.

25 EXAMPLE 18

26 A collagen sheet prepared as in Example 10 was rolled  
27 into a tube and then stabilized by tanning using a solution  
28 of 0.01% glutaraldehyde for 18 hours.

29 EXAMPLE 19

30 A collagen sheet prepared as in Example 10 was dried by  
31 critical point drying using liquid carbon dioxide.

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13 Separation of native types I, II and III by differential  
14 precipitation.

15       Modifications and adaptations may be made to the  
16 above described without departing from the spirit and scope  
17 of this invention which includes every novel feature and  
18 combination of features disclosed herein.

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1            CLAIMS:

2     1. Collagen in tactoid form obtained by forming an aqueous  
3 solution containing dissolved collagen and a water soluble  
4 or miscible polymer adapted to precipitate collagen out of  
5 solution in the form of tactoids.

6     2. A method of producing a collagen product comprising  
7 forming an aqueous solution containing dissolved collagen  
8 and a water soluble or miscible polymer adapted to  
9 precipitate the collagen out of solution in the form of  
10 tactoids.

11    3. A method of producing a collagen product as claimed in  
12 claim 2, wherein the pH of said solution is 3.5 - 10.

13    4. A method of producing a collagen product as claimed in  
14 claim 2, wherein the pH of said solution is 7 - 8.

15    5. A method of producing a collagen product as claimed in  
16 any one of claims 2 - 4, including forming the thus formed  
17 precipitate to a shape.

18    6. A method of producing a collagen product as claimed in  
19 any one of claims 2 - 5, including precipitating the  
20 collagen onto a pre-shaped substrate.

21    7. A method of producing a collagen product as claimed in  
22 claim 6, wherein the substrate has the form of a body part.

23    8. A method of producing a collagen product as claimed in  
24 claim 6, wherein the substrate is itself formed of collagen  
25 in the form of tactoids.

26    9. A method of producing a collagen product as claimed in  
27 claim 5, wherein prior to forming said precipitate to a  
28 shape the precipitate is permitted to stand in said solution  
29 for a period of greater than 1 hour.

30    10. A method of producing a collagen product as claimed in  
31 claim 9, wherein the temperature of standing is from 0 -  
32 20°C.

33    11. A method of producing a collagen product as claimed in  
34 any one of claims 2 - 10, and including the step of  
35 chemically or biochemically stabilizing the collagen so  
36 formed.

37    12. A method of producing a collagen product as claimed in  
38 any one of claims 2 - 11, wherein the dissolved collagen is

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1 derived from cell or tissue culturing.

2 13. A method of producing a collagen product as claimed in  
3 any one of claims 2 - 12, wherein said water soluble or  
4 miscible polymer is selected from polyvinyl alcohol,  
5 polyethylene oxide, polyvinylpyrrolidinone, polyacrylamide,  
6 polyethylene glycol, polypropylene glycol, polyvinyl methyl  
7 ether, maleic anhydride copolymers and the like.

8 14. A method of producing a collagen product as claimed in  
9 any one of claims 2 - 12, wherein said water soluble or  
10 miscible polymer is selected from hydroxyethyl starches,  
11 methyl cellulose, hydroxymethyl cellulose, hydroxyethyl  
12 cellulose, hydroxypropyl cellulose or the like.

13 15. A method of producing a collagen product as claimed in  
14 any one of claims 2 - 12, wherein said water soluble or  
15 miscible polymer is selected from agarose, dextrans,  
16 dextrans, starches; pectins, alginates and the like.

17 16. Collagen as claimed in claim 1 and in admixture with a  
18 biologically active material.

19 17. Collagen as claimed in claim 1 and in the form of a  
20 synthetic body part.

21 18. Collagen as claimed in claim 1 and precipitated onto a  
22 shaped substrate.

23 19. Collagen as claimed in claim 17 and in the form of a  
24 sheet or tube.

25 20. Collagen as claimed in claim 1 and in the form of a  
26 slurry or paste.

27 21. Collagen as claimed in claim 20 and containing at least  
28 10 mgm/ml of collagen.

29 22. A method of producing a collagen product substantially  
30 as hereinbefore described with reference to any one of the  
31 Examples.

32 23. Collagen in tactoid form substantially as hereinbefore  
33 described with reference to any one of the Examples.

34 24. The articles, things, parts, elements, steps, features,  
35 methods, processes, compounds and compositions referred to  
36 or indicated in the specification and/or claims of the  
37 application individually or collectively, and any and all  
38 combinations of any two or more of such.

# INTERNATIONAL SEARCH REPORT

International Application No PCT/AU 87/00038

## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) \*

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl. 4 A61L 27/00; C07K 15/12, 15/20; C08J 3/14; C08L 89/00, 89/06

## II. FIELDS SEARCHED

Minimum Documentation Searched ?

Classification System	Classification Symbols
IPC	A61L 27/00; C07K 15/12, 15/20; C08J 3/14; C08L 89/00, 89/06

Documentation Searched other than Minimum Documentation  
to the Extent that such Documents are Included in the Fields Searched \*

AU : IPC as above, Australian Classification 47.72

## III. DOCUMENTS CONSIDERED TO BE RELEVANT\*

Category *	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. 13
A	AU,A, 33803/84 (JOHNSON AND JOHNSON) 18 April 1985 (18.04.85)	
A	AU,A, 47013/85 (COLLAGEN CORPORATION) 13 March 1986 (13.03.86)	
A	AU,A, 51602/85 (COLLAGEN CORPORATION) 17 July 1986 (17.07.86)	
A	US,A, 4585797 (CIOCA) 29 April 1986 (29.04.86)	
A	US,A, 4407787 (STEMBERGER) 4 October 1986 (04.10.86)	
A	US,A, 4264155 (MIYATA) 28 April 1981 (28.04.81)	

\* Special categories of cited documents: 18

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"A" document member of the same patent family

## IV. CERTIFICATION

Date of the Actual Completion of the International Search

10 April 1987 (10.04.87)

Date of Mailing of this International Search Report

(05.05.87) 5 MAY 1987

International Searching Authority

Australian Patent Office

Signature of Authorized Officer

*Q menz* L. MENZ

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON  
INTERNATIONAL APPLICATION NO. PCT/AU 87/00038

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Members			
AU 33803/84	EP 140596 US 4614794	GB 8326542 ZA 8407780	GB 2148901	
AU 47013/85	EP 174175	JP 61137826	US 4557764	
AU 51602/85	EP 187014	JP 61210040	US 4600533	
US 4585797	AR 230006 FR 2503561	BR 8200482 US 4591501	DE 3204512 ZA 8108919	
US 4407787	CA 1167726 HK 534/86	DE 3037513 JP 58041559	EP 49469	
US 4264155	JP 56011430	US 4268131		

END OF ANNEX